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**Animal Experiment**

# Iron overload can induce mild copper deficiency

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## Summary

Dietary copper in the U.S. often is lower than that proved insufficient for men and women under controlled conditions. Iron overload can have adverse effects on copper nutriture and can produce cardiac disease in people. The hypothesis that iron can interfere with copper utilization to produce adverse effects related to cardiovascular function was tested.

Rats were fed a diet high in iron and marginal, but not deficient in copper for comparison with similar diets containing iron at the recommended amount. Copper and iron were measured by atomic absorption spectroscopy; cholesterol was measured by fluorescence, ceruloplasmin was measured by oxidase activity and hematology was done by an automated cell counter. When dietary copper was 2.0 mg/kg of diet, high iron decreased ($p<0.008$) cardiac and hepatic copper, plasma copper and ceruloplasmin, and increased ($p<0.02$) cardiac weight, hepatic iron and plasma cholesterol. When dietary copper was increased to 2.5 mg/kg, copper in heart and plasma decreased ($p<0.04$) and hepatic iron increased ($p=0.001$) with high iron but other effects disappeared. No harmful changes in hematology, such as hematocrit, mean corpuscular volume, etc. were found. High iron increased the dietary copper requirement of the animals. People with iron overload may benefit from copper supplementation, particularly if they habitually consume a diet low in copper.

***Keywords:*** Cardiomyopathy, cholesterol, copper, hemochromatosis, iron, iron overload.
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## Introduction

There seems to be little doubt that excessive tissue iron can have severe, pathological consequences. Iron can accumulate in several ways (1, 2), but the organs most affected can be quite variable (3). Cardiac disease is prominent among people with hemochromatosis (3, 4); one of several iron storage diseases (2). Iron is known to interfere with copper utilization (5, 6), but the phenomenon has not been studied extensively.

With mean daily intakes of copper of approximately 1.0 mg and the estimated safe and adequate daily intake of 1.5 to 3.0 mg (7), it seems likely that some people eat too little copper. The highest daily intake of copper proved insufficient for adults in a controlled trial to date is 1.02 mg (8); approximately 30% of daily diets contain less than that amount (9). Cardiomyopathy (10, 11) and hypercholesterolemia (12, 13) are prominent among the newer effects of copper deficiency that have been discovered.

These experiments were designed to test the hypothesis that a high intake of iron can interfere with the utilization of copper when intakes are marginal. Cholesterolemia and cardiac weight are among the more important of the measurements made.

## Materials and Methods

Male, weanling rats of the Sprague-Dawley strain (Sasco, Omaha, NE[1]) were divided into two equal groups of 15 or 14 by mean weight 51 or 48g in experiments one and two, respectively. They had free access to a purified diet based on sucrose (62%), egg white protein (20%) and corn oil (10%) containing all nutrients known to be essential for rats. This diet has been in use for more than 20 years (12) with minimal modification (14). For the present experiments two batches of Jones-Foster salt mix without added copper or zinc were made commercially (ICN, Irvine, CA) to provide each kg of diet with either 217 mg or 35 mg of iron. The former concentration is usual for diets made with this salt mix; the latter is the amount suggested for rats by the National Research Council (15). Finely ground zinc acetate was added to increase the amount of zinc in each diet by 13 mg/kg.

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Copper was measured in diet (and organs) by atomic absorption spectroscopy following destruction of organic matter with nitric and sulfuric acids and hydrogen peroxide (16). Then enough finely ground cupric sulfate was added to the diet to give a concentration of either 2.0 (experiment one) or 2.5 (experiment two) mg/kg. Dietary copper was increased in the second experiment to determine whether higher copper can lessen the adverse effects of high iron found in the first. The lower concentration of copper has been shown to be marginal for cardiovascular health (17); purified diets for rats usually contain 5 mg/kg (15).

Rats were killed at appropriate times by intoxication with sodium pentobarbital and pneumothorax for collection of samples. Anticoagulated blood (ethylenediaminetetraacetate) was obtained by cardiac puncture. Cholesterol and ceruloplasmin were measured by fluorescence (18) and oxidase activity (19), respectively. Erythrocyte count, hematocrit, hemoglobin and indices were measured and calculated by an automated cell counter (Cell-Dyn 3500, Abbott Laboratories, Santa Clara, CA). The first experiment was completed after 42 days when hypercholesterolemia was detected in blood collected from the tail vein. No difference in cholesterolemia between groups was found in three sequential bleedings in experiment two; rats were killed on the 96th day. Results were compared with the "t" test for unequal variances (20).

## Results

In experiment one, in which dietary copper as 2.0 mg/kg, there were no significant differences related to dietary iron in erythrocyte count, hematocrit, hemoglobin; mean corpuscular volume, or mean corpuscular hemoglobin, with group differences being less than 2.2%. Anemia was not detected. Mean corpuscular hemoglobin concentration increased slightly at the higher amount of dietary iron (32.5 ± 0.2 vs 33.4 ± 0.01, $p < 0.006$, mean ± SE).

The effects of dietary iron on other indices commonly altered by copper deficiency are contained in Table 1. Cardiac weight, hepatic iron and plasma cholesterol were increased by higher dietary iron; all other indices were decreased.

Data from the second experiment in which dietary copper was 2.5 mg/kg are contained in Table 2. No significant differences in hematology were found. In contrast to experiment one, dietary iron affected only cardiac copper, hepatic iron and plasma copper.

## Discussion

It seems clear that iron can interfere with copper utilization when dietary copper is marginal. Higher dietary iron decreased organ copper and ceruloplasmin and adversely increased measurements such as cardiac weight, hepatic iron and plasma cholesterol which can be minimized by adequate copper nutrition. There are some interesting contrasts between the two experiments, however.

Table 1. Copper indicators, 2.0 mg copper/kg[a]

| | Lower F | Higher Fe | p |
|---|---|---|---|
| Cardiac | | | |
| copper (μg/g) | 12.9 ± 1.1 | 7.4 ± 0.5 | <0.0002 |
| weight ( g) | 1.22± 0.02 | 1.38± 0.04 | <0.005 |
| Hepatic | | | |
| copper (μg/g) | 8.7 ± 0.55 | 5.6 ± 0.71 | <0.002 |
| iron (μg/g) | 301 ±25.9 | 648 ±52.8 | 0.0001 |
| Plasma | | | |
| cholesterol (mg/dl) | 127 ± 4.2 | 141 ± 3.9 | <0.02 |
| copper (μg/ml) | 0.53± 0.10 | 0.20± 0.05 | <0.007 |
| Serum | | | |
| ceruloplasmin (mg/dl) | 20.3 ± 5.19 | 4.0 ± 1.47 | <0.008 |

[a]$\bar{X}$±SE

Higher dietary iron induced an increase in plasma cholesterol in the first experiment, but had no effect on sequential measurements in the second experiment which lasted more than twice as long. The higher amount of dietary copper in the second experiment was insufficient to prevent hypercholesterolemia confirming a shorter experiment where 3 mg/kg produced lower cholesterol than 2 mg/kg (21). Mean values for plasma cholesterol of groups of rats fed this diet with sufficient copper generally do not exceed 110 mg/dl (21). That copper deficiency can induce hypercholesterolemia has been confirmed in numerous independent laboratories world-wide (for references see(14)) since discovery (12). It seems likely that the higher amount of copper prevented some of the ill effects of higher iron such as those on ceruloplasmin and organ copper.

At the lower amount of dietary copper, the mean ceruloplasmin of 20.3 mg/dl may be slightly lower than normal (21); higher dietary iron decreased this value by 80%. Ceruloplasmin was not lower than normal at the higher amount of dietary copper where it was unaffected by dietary iron. Plasma copper was decreased by higher dietary iron in both experiments.

Table 2 Copper indicators, 2.5 mg copper/kg[a]

| | Lower Fe | Higher Fe | P |
|---|---|---|---|
| Cardiac | | | |
| copper (μg/g) | 22.0 ± 1.5 | 17.3 ± 1.2 | <0.0025 |
| weight (g) | 1.41± 0.05 | 1.37± 0.04 | >0.5 |
| Hepatic | | | |
| copper (μg/g) | 10.8 ±.20 | 9.5 ± 0.73 | >0.1 |
| iron (μg/g) | 248 ± 11.7 | 524 ± 65.4 | 0.001 |
| Plasma | | | |
| cholesterol (mg/dl) | 155 ± 9.7 | 159 ± 11.4 | >0.7 |
| copper (μg/ml) | 1.5 ± 0.07 | 1.1 ± 0.16 | <0.04 |
| Serum | | | |
| ceruloplasmin (mg/dl) | 39.7 ± 2.8 | 34.1 ± 5.8 | >0.3 |

[a]$\bar{X}$±SE

Elvehjem and Sherman first noticed that copper deficiency increases hepatic iron (22). This phenomenon has been verified repeatedly; increased hepatic iron may be the most sensitive index of copper deficiency discovered to date (21, 23).

Cardiac enlargement in copper deficiency has been found numerous times in the last 30 years (23-25). Higher dietary iron increased heart weight only at the lower amount of dietary copper. In contrast, cardiac copper was decreased by higher dietary iron at both amounts of dietary copper. The decrease in cardiac copper induced by higher dietary iron was not an effect of dilution because the decline in copper (43%) was much greater than the increase in cardiac weight (13%) at the lower amount of dietary copper; higher dietary iron decreased cardiac copper without increasing cardiac size at the higher amount of dietary copper. Decreased cardiac copper can be associated with potentially harmful changes in cardiac anatomy without abnormally low values for plasma copper or ceruloplasmin (11).

It seems likely that if dietary copper had been increased to the 5 mg/kg usual for rats, the adverse effects of higher dietary iron would have been prevented. The fewer adverse effects found with dietary copper at 2.5 mg/kg in contrast to 2.0 mg/kg are consonant with iron having increased the dietary copper requirement.

That iron can interfere with copper utilization without anemia and without much effect, depending on amount of dietary copper, on usual indices of copper status may have implications for human health. Patients with iron overload may benefit from long term supplementation with copper. These patients should be evaluated with some of the newer, potentially more sensitive indices of copper status such as erythrocyte superoxide dismutase, leukocyte copper or platelet cytochrome c oxidase (26-28) or serum lysyl oxidase(29).

Pregnant women eating diets in the lowest tertile of copper content (less than 1 mg/day) will find it difficult, if not impossible, to avoid depletion of maternal copper stores (30). Iron supplements during pregnancy probably should be accompanied by copper supplements. Finland has abandoned (J.T. Salonen, personal communication) iron fortification of food, probably because of associations of iron with risk of ischemic heart disease (31-34). On the assumption that iron deficiency is more common than iron overload, it may be better to supplement people at risk for iron overload with copper and continue the fortification of food with iron.

Two mg copper/kg of diet exceeds the mean of 1.86 for dried human meals (35). The mean plus one standard deviation (2.48 mg/kg) for those data is almost exactly the higher amount of dietary copper in the present experiment. This higher amount thus exceeds the concentration of 84% of human diets. If the dietary requirement for copper of people with iron overload is generally greater than that of the healthy people for whom dietary recommendations are made (7), it will be hard for the latter to reach an adequate intake without supplementation.

The mechanism(s) by which iron overload produces cellular damage remains uncertain (36). Although phlebotomy improves survival and chelation improves organ function, some abnormalities associated with iron overload resist treatment. The heart may benefit most. Some endocrine function improves; arthritis generally is unaffected(36). Sometimes phlebotomy is not feasible as in severe thalassemia.

If iron overload induces copper deficiency in people as it does in rats, other benefits, besides cardiac, may occur from dietary supplementation with copper. Glucose intolerance occurs in deficient rats (37-39), can occur in Menkes' disease (40) and has been verified in men depleted of copper (41). Diabetes mellitus and cardiomyopathy are among the important causes of increased mortality in iron overload (4).

Bone disease in copper deficiency, first noticed in dogs by Baxter et al. (42, 43), reviewed by Underwood (44) and confirmed recently in pigs (45) and rats (46), has received less attention by copper researchers than cardiomyopathy. Bone disease in human iron overload seems to affect the joints (47) rather than the shafts of bones (3) which have received more attention in copper deficient animals. However, the dogs studied by Baxter can be seen to have leg deformities (48). Kaschin-Beck disease has both bone and joint deformities and has been associated with iron overload (1).

In summary, higher dietary iron can interfere with copper utilization, sometimes without peripheral evidence of effects on copper. Some of the adverse effects such as increased cholesterolemia and increased cardiac size can be diminished by a higher amount of dietary copper. The dietary requirement for copper of people with iron overload may exceed that of the general population. Laboratory evaluation should include newer tests of copper depletion. If iron overload induces copper deficiency in people as well as in rats, copper supplementation may be beneficial along with phlebotomy or in situations when phlebotomy is not useful. Cardiomyopathy, diabetes and joint disease may improve. Improvement of cardiac function of people containing too much iron is the most obvious potential benefit.

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